Pakistan J. Zool., vol. 49(6), pp 2193-2199, 2017. DOI: http://dx.doi.org/10.17582/journal.pjz/2017.49.6.2193.2199

Bioactivity of Extracts of *Foeniculum vulgare* and *Ocimum basilicum* against *Heterotermes indicola* (Wasmann)

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Article Information
Received 06 June 2017
Revised 26 July 2017
Accepted 07 August 2017
Available online 22 November 2017

Authors' Contribution
AA and MSA designed the project and supervised the work. MU performed the experimental work. AA helped in the collection of data. KZR and MID helped in the execution of the experimental work. AA and MID helped in handling the data. MU, AA and MSA wrote the article.

Key words
Bioactivity, *Foeniculum vulgare*, GC-MS, *Ocimum basilicum*, *Heterotermes indicola*.

ABSTRACT

As *Heterotermes indicola* (Wasmann) is known to cause considerable damage to wood work in buildings. Efficacy of extracts of *Foeniculum vulgare* and *Ocimum basilicum* was studied against this termite. Chemical composition of plant extracts by chromatography-mass spectrometry (GC-MS) revealed five different compounds in *F. vulgare*: Piperidine, 3-isopropyl, Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl, Benzaldehyde, 4-methoxy, Estragole, 11-Octadecenoic acid, methyl ester and 9-Octadecenoic acid ethyl ester. Whereas nine different compounds were identified in extracts of *O. basilicum*. These were: 1-Isopropyl-2, 2-dimethylpropylideneamine, Camphor, Naphthalene, Thymol, 1,5,5- Trimethyl-6-x methylene-cyclohexene, Hexadecanoic acid, methyl ester, 9,12,15 Octadecatrienoic acid, methyl ester, 8, 11, 14-Eicosatrienoic acid and 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester. Extracts of both the plants were found repellent to this termite. LT_{50} values of *O. basilicum* and *F. vulgare* against *Heterotermes indicola* were 60.91 and 115.9 h, respectively.

INTRODUCTION

Termites are abundant in tropical region and are very devastating to wood (Harris, 1971; Ajayi *et al*., 2012). *Heterotermes* spp. are structure-infesting termites that account for a significant proportion of damage wherever they occur and are confined to their respective climatic zones by the limits of soil moisture and temperature (Emerson, 1971; Saljoqi *et al*., 2012).

With the increasing spread of termite infestation, there is an increased need to find out human and environment safe treatments (Meepagala *et al*., 2006). Many attempts have been made in the field and in the laboratory to exploit the termite activity by plant extracts. Some plant species have been used in the past to explore its anti- termite activity (Adams *et al*., 1988). There is a growing interest in natural toxic substances from plants (Chang *et al*., 2001; Elango *et al*., 2012). Secondary metabolites like alkaloids, chromenes, coumarins and terpenoids, especially monoterpenoids are produced by the plants for their defense mechanism. These secondary metabolites have been evaluated for their domestic pests controlling properties. Attention towards oils was renewed in the 1990s with increasing expression of their fumigant and contact insecticidal activities for an extensive array of insects (Isman, 2000; Koul *et al*., 2008).

The harmful effects of phytochemicals against insects are manifested in numerous ways like inhibition of calling behavior (Khan and Saxena, 1986; Ahmed *et al*., 2011), delayed growth (Breuer and Schmidt, 1995; Ahmed *et al*., 2011), toxicity (Hiremath *et al*., 1997; Ahmed *et al*., 2011), avoidance of oviposition (Zhao *et al*., 1998; Ahmed *et al*., 2011), suppression in feeding (Wheeler and Isman, 2001; Ahmed *et al*., 2011) and drop of fertility (Muthukrishnan and Pushpalatha, 2001; Ahmed *et al*., 2011).

Present studies were undertaken to assess the toxic potential of the extracts of *F. vulgare* (fennel) and *O. basilicum* (niazbo) against *H. indicola*. Our objectives were (i) ethanolic extraction of selected plant seeds using soxhlet extractor and (ii) structural characterization of compounds of seeds extracts through GC-MS.

MATERIALS AND METHODS

Collection of termites

Termite workers and soldiers of species *H. indicola* (Wasmann) were collected from old trees of *Populus euramericana* from Lahore. The termites were maintained for at least 1 week on water soaked filter papers and 5 g

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0030-9923/2017/0006-2193 $ 9.00/0

oven dried soil in each Petri-plate.

Seeds collection

Seeds of locally used medicinal plants, *F. vulgare* (Saunf/Fennel) and *O. basilicum* (Niazbo) were purchased from local market.

Preparation of extracts

The seeds of the medicinal plants were ground into fine powder using a grinder. Twenty grams of each seed powder was taken separately for extraction in Soxhlet extractor with 200 ml of ethanol. Rotary evaporator was used to obtain dried residues and stored in refrigerator for making stock solution. Stock solution was prepared for each plant extract by taking 1 g dried extract in 10ml of absolute ethanol to get a solution of 10% concentration. Concentrations used were10%, 5% and 3%.

Gas chromatography/mass spectrometry

All seed samples were analyzed by gas chromatography coupled with mass spectrometry. The gas chromatography conditions include a temperature range of 50 to 250^{o}C with 4^{o}C/min, with a solvent delay of 5 min. The temperature of injector was maintained at 250^{o}C. Helium was used as an inert gas with a flow rate of 1.0 mL/min. and the volume of injected sample in the split less mode was 2μL. The MS conditions were the following: ionization energy, 70 eV; quadrupole temperature 100^{o}C; scanning velocity, 1.6 scans/s; weight range, 40-500 amu.

The percent composition of the samples was calculated. The qualitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the spectra library NIST 98 (USA National Institute of Science and Technology software).

Anti-termitic assay

Circular filter papers were cut and placed at the bottom of each sterilized glass Petri plate. Each filter paper was soaked with 0.5 ml of the 10%, 5% and 3% extracts concentrations. Petri plates containing filter paper were dried at ambient temperature. Fifty workers and five soldiers of *H. indicola* were added in each Petri plate. Observations were taken after every 2 h up to 12 h. Data for the mortality of the termite were recorded after an interval of 12 h up to 96 h.

$$\text{Mortality rate \%} = \frac{\text{No. of dead termites after test}}{\text{No. of initial termites used in test}} \times 100$$

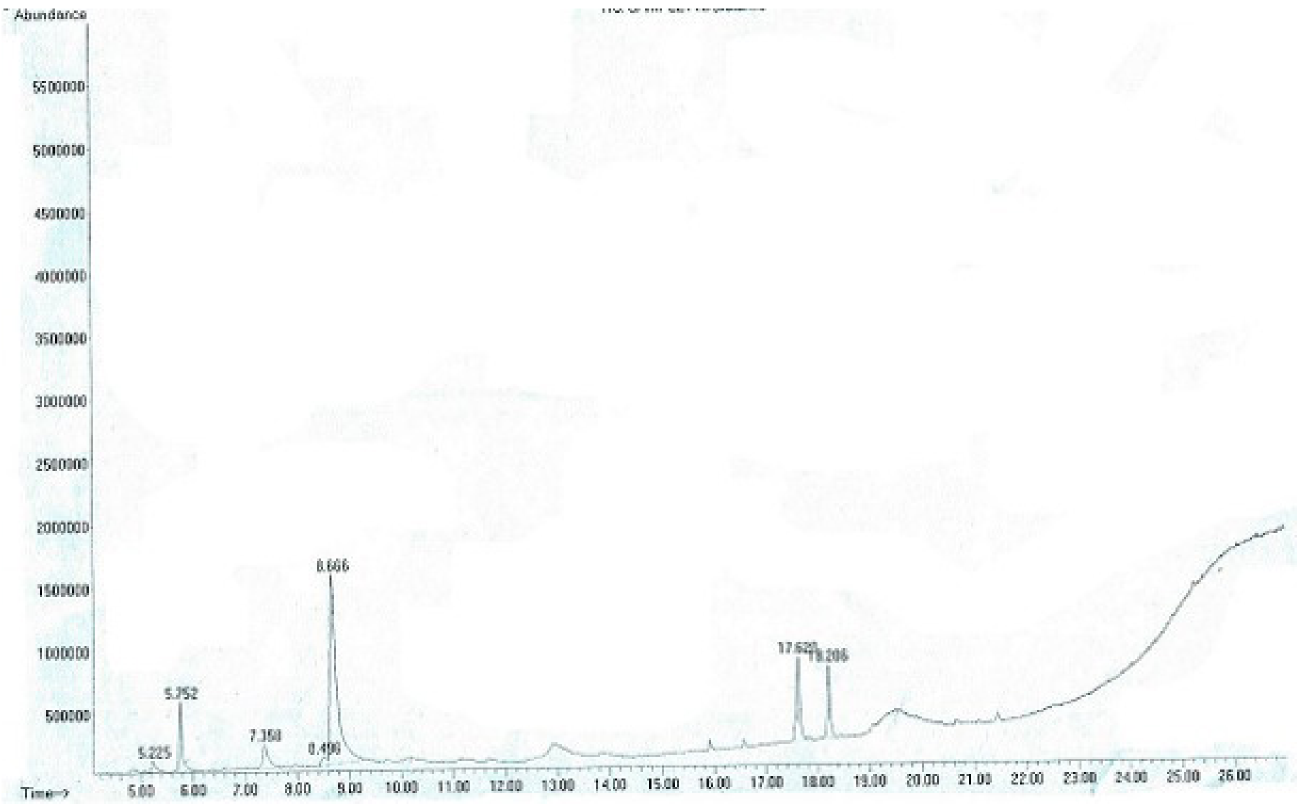
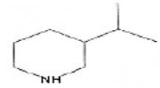
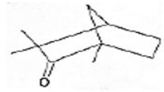
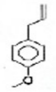
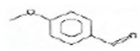
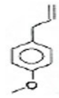
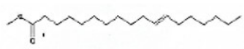
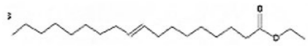


Fig. 1. Compounds identified by GC-MS analysis in *F. vulgare.*

Table I.- Phytocompounds identified in ethanolic extract of *F. vulgare*.

Retention time Minutes	Phytocompounds	Relative percentage composition (%)	Structural formulae
5.225	Piperidine, 3-isopropyl	3.79	
5.752	Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl	14.63	
7.358	Estragole	12.35	
8.496	Benzaldehyde, 4-methoxy	4.19	
8.666	Estragole	100	
17.620	11-Octadecenoic acid, methyl ester	20.84	
18.206	9-Octadecenoic acid ethyl ester	15.09	

Repellency assay

For the estimation of repellency filter papers of 9cm in diameter were cut into two equal halves. One half of each filter paper was treated with 10%, 5% and 3% concentration of extracts and second half was treated with distilled water (untreated). The two halves were placed into the Petri dishes with a cut space in the middle. A total of 10 termites were released into the middle space. Repellency was noted after every 15 min by counting the number of termites on treated (T) and untreated (UT) filter paper discs and experiment was conducted for 2 h. Three replicates were prepared for each concentration of all four plant extracts. A treatment concentration was considered repellent when 21 (sum of three replicates) of 30 termites were present on untreated filter paper for five consecutive readings.

Statistical analysis

Mortality percentage of termites was calculated and analyzed by using one way Anova values of $P<0.05$ were considered significant statistically. LT_{50} was calculated by using Probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Extracts of *F. vulgare* and *O. basilicum* which are well known for their medicinal importance had an impact of termite survival also. LT_{50} value of termites exposed to extracts of *O. basilicum* was 60.91 h; and the extract was also repellent. Extracts of *F. vulgare* was slightly less effective with LT_{50} value as 115.9 h. But, its extract has also termite repellent attribute.

As regard Chemical composition/GC-MS analysis of the extract of *F. vulgare* revealed the presence of piperidine, bicyclo[2.2.1]heptan-2-one, estragole, benzaldehyde, 11-octadecenoic acid and 9-octadecenoic acid (Fig. 1, Table I). The least termiticidal effect was found in *F. vulgare* which was 37% against *H. indicola*. *Foeniculum vulgare* Mill commonly called fennel has been used in traditional medicine for a wide range of ailments related to digestive, endocrine, reproductive, and respiratory systems. *Foeniculum vulgare* remains to be the most widely used herbal plant. It has been used for more than forty types of disorders. Phytochemical studies have shown the presence of numerous valuable compounds, such as volatile compounds, flavonoids, phenolic compounds, fatty acids, and amino acids. Data indicate their efficacy in several *in vitro* and *in vivo* pharmacological properties such as antimicrobial, antiviral, anti-inflammatory, antimutagenic, antinociceptive, antipyretic, antispasmodic, antithrombotic, apoptotic, cardiovascular, chemomodulatory, antitumor, hepatoprotective, hypoglycemic, hypolipidemic, and memory enhancing property (Badgujar *et al*., 2014). Abbas *et al*. (2013) conducted the study on EtOH extracts of fifteen medicinal plant seed which showed excellent antitermitic activity and the LT_{50} of *Foeniculum vulgare, Peganum harmala, Psoralea corylifolia, Ricinus communis, Croton tiglium, Mentha* species, *O. sativum* and *Capsicum frutescens* was found lower than 10 h.

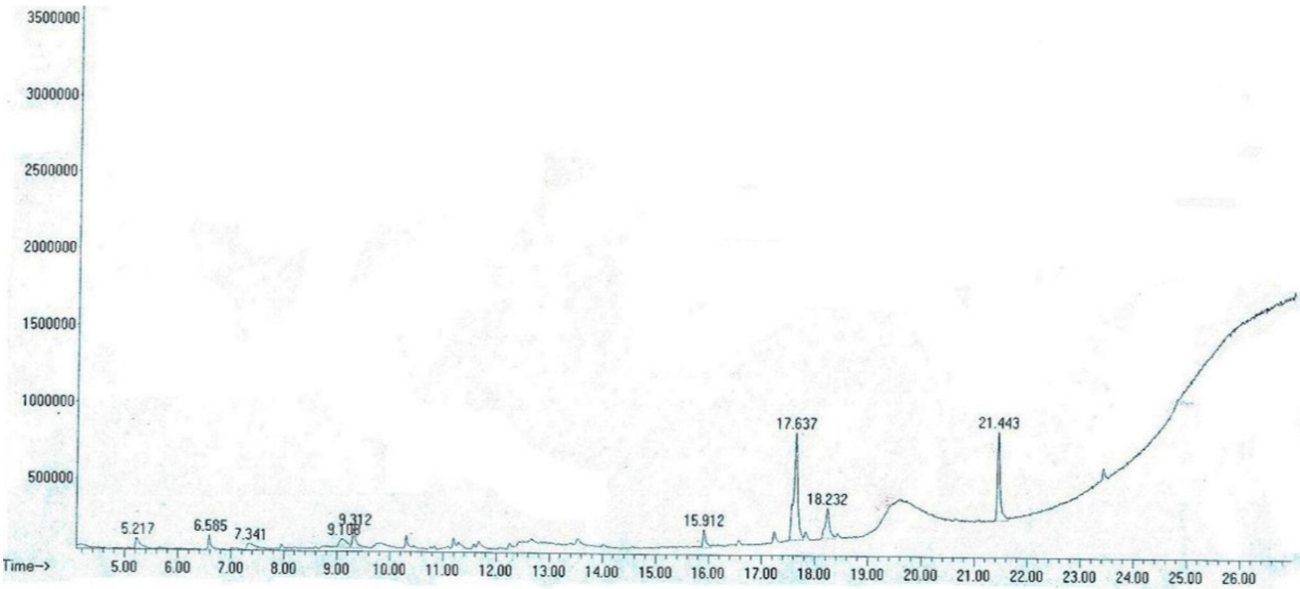
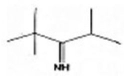
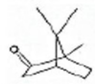
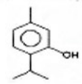
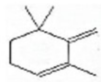
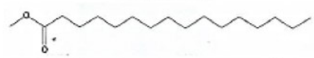
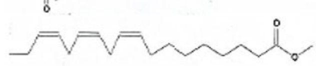
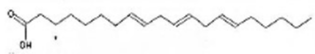
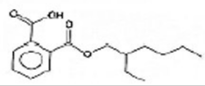


Fig. 2. Compounds identified by GC-MS analysis in *O. basilicum*.

Table II.- Phytocompounds identified in ethanolic extract of *O. basilicum*.

Retention time Minutes	Phytocompounds	Relative percentage composition (%)	Structural formulae
5.217	1-Isopropyl-2, 2-dimethylpropylideneamine	11.82	
6.585	Camphor	9.40	
7.341	Naphthalene	11.73	
9.108	Thymol	14.56	
9.312	1,5,5-Trimethyl-6-methylene-cyclohexene	15.02	
15.912	Hexadecanoic acid, methyl ester	12.32	
17.637	9,12,15-Octadecatrienoic acid, methyl ester	100	
18.232	8, 11, 14-Eicosatrienoic acid	30.03	
21.443	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) esters	56.51	

The GCMS analysis of *O. basilicum* revealed the presence of components such as dimethylpropylamine, camphor, naphthalene, thymol, methylene cyclohexene, hexadecanoic acid, octadecatrienoic acid, eicosatrienoic acid and benzene dicarboxylic acid as shown in Figure 2 and Table II. A related study was conducted by Govindarajan *et al*. (2013) on the toxicity of mosquito larvicidal activity of leaf essential oil and their major chemical constituents from *Ocimum basilicum* against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus*. GC–MS revealed that the essential oil of *O. basilicum* contained 20 compounds. The major chemical components identified were linalool (52.42%), methyl eugenol (18.74%) and 1, 8-cineol (5.61%). The essential oil had a significant toxic effect against late third-stage larvae of *Cx. tritaeniorhynchus, Ae. albopictus* and *An. subpictus* with an LC_{50} values of 14.01, 11.97 and 9.75 ppm and an LC_{90} values of 23.44, 21.17 and 18.56 ppm, respectively. The active ingredients in the essential oils were separated and identified by TLC and GC-MS in a study conducted by Nour *et al*. (2012) essential oils from two basil (*O. basilicum*) accessions were tested in a laboratory bioassay for larvicidal activity against third instar *A. aegypti* larvae. Approximately 13 compounds (>1%) were detected; most compounds were oxygenated monoterpenes. Linalool, geraniol, geranial, methyl chavicol and eugenol were active components against *A. aegypti* larvae.

The studied extracts of *O. basilicum* and *F. vulgare* contained biological active compound which showed greater potential against *H. indicola*. Control was established as preliminary test to check the termiticidal function of extracts with distilled water filter papers. Control was non toxic as most of the termites were remained alive for long time. Subsequently lethal time was determined as LT_{50} by Probit analysis. In our findings, *O. basilicum* showed high anti termitic activity with LT_{50} of 10%, 5% and 3% concentrations as 60.91, 121.2 and 154.6 h, respectively. 83% termites were killed in 10% concentration of *O. basilicum* which was reduced in *F. vulgare* to 37%. LT_{50} of *F. vulgare* extracts was 115.9, 162.6 and 184.7 h, respectively as shown in Figure 3.

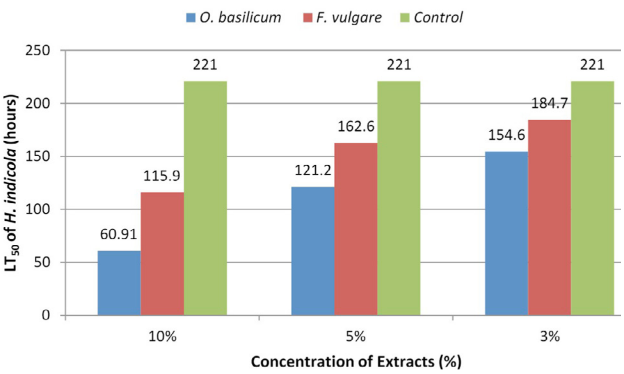


Fig. 3. LT_{50} of all concentrations of Extracts against *H. indicola.*

When termite workers exposed to 10%, 5% and 3% concentrations of *O. basilicum* along with untreated filter papers. Result showed that 3% concentration was non repellent to *H. indicola* as less than 21 termites were present on untreated filter paper while 10% and 5% concentrations were found to be repellent, however all concentrations of *F. vulgare i.e.* 10%, 5% and 3% were repellent against *H. indicola* as shown in Figure 4.

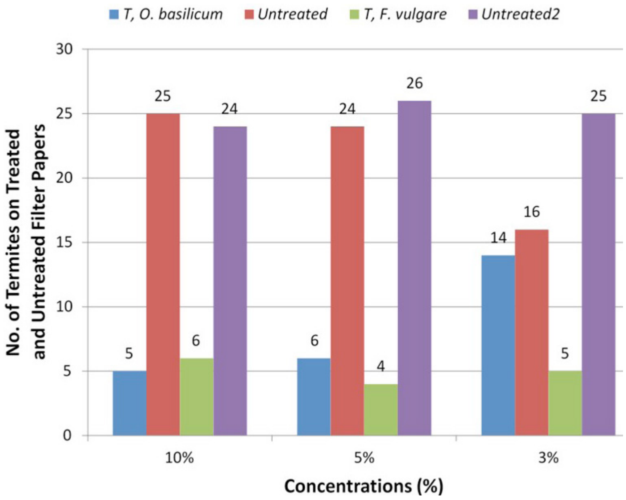


Fig. 4. Repellency test of *O. basilicum* and *F. vulgare* against *H. indicola.*

Termites as being devastating organisms to the wood and related products, a variety of techniques have been used to control them; the use of artificial pesticides is the most common practice. These chemicals are suspect to be absolutely effective against termites, however, they proved to be obstinate in nature and become part of our food chain thereby leading to process of magnification. If there are any leakages after heavy rain these insecticides cause serious environmental hazards (Logan *et al*., 1990; Martius, 1998; Jamil *et al*., 2005; Qureshi *et al*., 2012). Many attempts have been made in field and laboratory to exploit anti termitic activities of plants extracts. Some plant species were used in past to explore their anti-termite activities, insecticidal properties and anti-feedant activities and contain certain chemicals that reduce termite growth or kill them (Adams *et al*., 1988; Qureshi *et al*., 2015). In this study the toxic potential of *O. basilicum* and *F. vulgare* was investigated against *H. indicola.*

Ethanol extracts of *F. vulgare* and *O. basilicum* have a potential to be used for termite control. Both the extracts were found toxic against *H. indicola*

CONCLUSION

Ethanol extracts of *F. vulgare* and *O. basilicum* have a potential to be used for termite control. Both the extracts were found toxic against *H. indicola.* Likewise, these extracts must also be tried against termite species in different ecological zones of Pakistan for understanding better control and management of other termite species.

ACKNOWLEDGEMENTS

We are grateful to the Department of Zoology, University of the Punjab, Lahore, Pakistan for providing laboratory facilities for research work which is greatly appreciated and acknowledged.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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